

## WHAT IS CLAIMED IS:

1. A method of inhibiting HCV replication in an HCV infected cell comprising the step of providing to said cell an effective amount of a compound  
 5 that inhibits NS2/3 autocleavage.

2. The method of claim 1, wherein said compound is selected from the group consisting of:  
 an HCV inhibitor polypeptide comprising an NS4A fragment at  
 10 least about 11 amino acids in length, wherein said fragment can inhibit autocleavage of NS2/3;  
 a pharmaceutically acceptable salt of said HCV inhibitor polypeptide; and  
 a prodrug thereof.

3. The method of claim 1, wherein compound is selected from the group consisting of:  
 a polypeptide having the structure:

20  $Z^1-Y^1_m-X^1X^2X^3X^4X^5GX^6X^7X^8X^9X^{10}-Y^2_n-Z^2$

wherein  $X^1$  is either serine, cysteine, or threonine;  
 $X^2$  is either valine, leucine, or isoleucine;  
 $X^3$  is either valine, leucine, isoleucine, serine, cysteine or threonine;  
 25  $X^4$  is either valine, leucine, or isoleucine;  
 $X^5$  is either valine, leucine, or isoleucine;  
 $X^6$  is either lysine, arginine, or histidine;  
 $X^7$  is either valine, leucine, or isoleucine;  
 $X^8$  is either aspartic acid, glutamic acid, valine, leucine, isoleucine, lysine, arginine,  
 30 or histidine;  
 $X^9$  is either valine, leucine, or isoleucine;  
 $X^{10}$  is either serine, cysteine, threonine, asparagine, glutamine, aspartic acid, or glutamic acid;  
 each  $Y^1$  is an independently selected amino acid,  
 35 each  $Y^2$  is an independently selected amino acid,

Z<sup>1</sup> is an optionally present protecting group covalently joined to Y<sup>1</sup>,  
 Z<sup>2</sup> is an optionally present protecting group covalently joined to Y<sup>2</sup>,  
 m is from 0 to 300, and  
 n is from 0 to 300,

5 a pharmaceutically acceptable salt of said polypeptide; and  
 a prodrug thereof.

4. The method of claim 3, wherein m is from 0 to 25 and n is from  
 0 to 25.

10 5. The method of claim 4, wherein said compound is said  
 polypeptide or a pharmaceutically acceptable salt thereof.

6. The method of claim 1, wherein said compound is selected  
 15 from the group consisting of:  
 KGSVVIVGRILSGRK (SEQ. ID. NO. 16),  
 Ac-GGSVVIVGRILSGRK (SEQ. ID. NO. 18),  
 GGSVVIVGRILSGRG (SEQ. ID. NO. 19),  
 KKGSVVIVGRILSGRPAIVPRR-NH<sub>2</sub> (SEQ. ID. NO. 20), and  
 20 KKGSVVIVGRILSGRPAIVPDRELLYQEFDE (SEQ. ID. NO. 21),  
 or a pharmaceutically acceptable salt thereof.

7. A method of inhibiting HCV replication in an HCV infected  
 cell comprising the step of introducing into said cell an effective amount of a nucleic  
 25 acid comprising a nucleotide sequence encoding for a polypeptide comprising an  
 NS4A fragment at least about 11 amino acids in length, wherein said fragment  
 inhibits autocleavage of NS2/3.

8. The method of claim 7, wherein said nucleic acid is an  
 30 expression vector.

9. A method of inhibiting HCV replication in an HCV infected  
 cell comprising the step of introducing into said cell an effective amount of a nucleic

acid comprising a nucleotide sequence encoding for a polypeptide having the structure:



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wherein  $X^1$  is either serine, cysteine, or threonine;

$X^2$  is either valine, leucine, or isoleucine;

$X^3$  is either valine, leucine, isoleucine, serine, cysteine or threonine;

$X^4$  is either valine, leucine, or isoleucine;

10  $X^5$  is either valine, leucine, or isoleucine;

$X^6$  is either lysine, arginine, or histidine;

$X^7$  is either valine, leucine, or isoleucine;

$X^8$  is either aspartic acid, glutamic acid, valine, leucine, isoleucine, lysine, arginine, or histidine;

15  $X^9$  is either valine, leucine, or isoleucine;

$X^{10}$  is either serine, cysteine, threonine, asparagine, glutamine, aspartic acid, or glutamic acid;

each  $Y^1$  is an independently selected amino acid;

each  $Y^2$  is an independently selected amino acid;

20  $m$  is from 0 to 300; and

$n$  is from 0 to 300.

10. The method of claim 9, wherein said nucleic acid is an expression vector.

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11. The method of claim 10, wherein  $m$  is from 0 to 25, and  $n$  is from 0 to 25.

12. A method of treating a patient for HCV comprising the step of inhibiting NS2/3 autocleavage.

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13. The method of claim 12, wherein said patient is a human patient and said method further comprises the step of identifying said patient as infected with HCV prior to said inhibiting.

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14. The method of claim 12, wherein said step of inhibiting NS2/3 autocleavage is achieved using an effective amount of a compound selected from the group consisting of:

- a polypeptide comprising an NS4A fragment at least about 11 amino acids in length;
- a pharmaceutically acceptable salt of said polypeptide; and
- a prodrug thereof.

15. The method of claim 12, wherein said step of inhibiting NS2/3 autocleavage is achieved using an effective amount of a compound selected from the group consisting of:

a polypeptide having the structure:



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wherein  $X^1$  is either serine, cysteine, or threonine;

$X^2$  is either valine, leucine, or isoleucine;

$X^3$  is either valine, leucine, isoleucine, serine, cysteine or threonine;

$X^4$  is either valine, leucine, or isoleucine;

20  $X^5$  is either valine, leucine, or isoleucine;

$X^6$  is either lysine, arginine, or histidine;

$X^7$  is either valine, leucine, or isoleucine;

$X^8$  is either aspartic acid, glutamic acid, valine, leucine, isoleucine, lysine, arginine, or histidine;

25  $X^9$  is either valine, leucine, or isoleucine;

$X^{10}$  is either serine, cysteine, threonine, asparagine, glutamine, aspartic acid, or glutamic acid;

each  $Y^1$  is an independently selected amino acid,

each  $Y^2$  is an independently selected amino acid,

30  $Z^1$  is an optionally present protecting group covalently joined to  $Y^1$ ,

$Z^2$  is an optionally present protecting group covalently joined to  $Y^2$ ,

$m$  is from 0 to 300, and

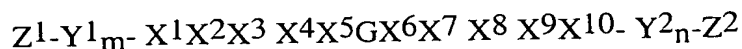
$n$  is from 0 to 300,

- a pharmaceutically acceptable salt of said polypeptide; and
- a prodrug thereof.

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16. A method of inhibiting or preventing HCV replication in a patient comprising the step of treating said patient with an effective amount of a compound selected from the group consisting of:

- 5 a polypeptide that either comprises an NS4A fragment at least about 11 amino acids in length able to inhibit NS2/3 autocleavage or has the structure:



- 10 wherein  $X^1$  is either serine, cysteine, or threonine;  
 $X^2$  is either valine, leucine, or isoleucine;  
 $X^3$  is either valine, leucine, isoleucine, serine, cysteine or threonine;  
 $X^4$  is either valine, leucine, or isoleucine;  
 $X^5$  is either valine, leucine, or isoleucine;  
15  $X^6$  is either lysine, arginine, or histidine;  
 $X^7$  is either valine, leucine, or isoleucine;  
 $X^8$  is either aspartic acid, glutamic acid, valine, leucine, isoleucine, lysine, arginine, or histidine;  
 $X^9$  is either valine, leucine, or isoleucine;  
20  $X^{10}$  is either serine, cysteine, threonine, asparagine, glutamine, aspartic acid, or glutamic acid;  
each  $Y^1$  is an independently selected amino acid,  
each  $Y^2$  is an independently selected amino acid,  
 $Z^1$  is an optionally present protecting group covalently joined to  $Y^1$ ,  
25  $Z^2$  is an optionally present protecting group covalently joined to  $Y^2$ ,  
m is from 0 to 300, and  
n is from 0 to 300,

a pharmaceutically acceptable salt of said polypeptide; and  
a prodrug thereof.

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17. The method of claim 16, wherein said patient is a human infected with HCV.

18. The method of claim 17, wherein said compound is said  
35 polypeptide or a pharmaceutically acceptable salt thereof.

19. The method of claim 17, wherein said compound is selected from the group consisting of:

KGSVVIVGRILSGRK (SEQ. ID. NO. 16),

5 Ac-GGSVVIVGRILSGRK (SEQ. ID. NO. 18),

GGSVVIVGRILSGRG (SEQ. ID. NO. 19),

KKGSVVIVGRILSGRPAIVPRR-NH<sub>2</sub> (SEQ. ID. NO. 20), and

KKGSVVIVGRILSGRPAIVPDRELLYQEFDE (SEQ. ID. NO. 21),

or a pharmaceutically acceptable salt thereof.

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20. A method of inhibiting or preventing HCV replication in a patient comprising the step of administering to said patient an effective amount of a nucleic acid comprising a nucleotide sequence encoding for a polypeptide comprising an NS4A fragment at least about 11 amino acids in length, wherein said fragment

15 inhibits autocleavage of NS2/3.

21. The method of claim 20, wherein said nucleic acid is an expression vector.

20 22. A method of inhibiting or preventing HCV replication in a patient comprising the step of administering to said patient an effective amount of a nucleic acid comprising a nucleotide sequence encoding for a polypeptide having the structure:

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$$Y^1_m - X^1 X^2 X^3 X^4 X^5 G X^6 X^7 X^8 X^9 X^{10} - Y^2_n$$

wherein X<sup>1</sup> is either serine, cysteine, or threonine;

X<sup>2</sup> is either valine, leucine, or isoleucine;

X<sup>3</sup> is either valine, leucine, isoleucine, serine, cysteine or threonine;

30 X<sup>4</sup> is either valine, leucine, or isoleucine;

X<sup>5</sup> is either valine, leucine, or isoleucine;

X<sup>6</sup> is either lysine, arginine, or histidine;

X<sup>7</sup> is either valine, leucine, or isoleucine;

X<sup>8</sup> is either aspartic acid, glutamic acid, valine, leucine, isoleucine, lysine, arginine,

35 or histidine;

X<sup>9</sup> is either valine, leucine, or isoleucine;

X<sup>10</sup> is either serine, cysteine, threonine, asparagine, glutamine, aspartic acid, or glutamic acid;

each Y<sup>1</sup> is an independently selected amino acid;

5 each Y<sup>2</sup> is an independently selected amino acid;

m is from 0 to 300; and

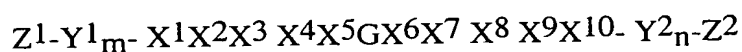
n is from 0 to 300.

10 23. The method of claim 22, wherein said nucleic acid is an expression vector.

24. The method of claim 22, wherein m is from 0 to 25 and n is from 0 to 25.

15 25. A compound selected from the group consisting of:  
a pharmaceutically acceptable salt of a HCV inhibitor polypeptide, wherein said HCV inhibitor polypeptide comprises an NS4A fragment at least about 11 amino acids in length and can inhibit autocleavage of NS2/3; and  
a prodrug thereof.

20 26. A compound selected from the group consisting of:  
a polypeptide having the structure:



25 wherein X<sup>1</sup> is either serine, cysteine, or threonine;

X<sup>2</sup> is either valine, leucine, or isoleucine;

X<sup>3</sup> is either valine, leucine, isoleucine, serine, cysteine or threonine;

X<sup>4</sup> is either valine, leucine, or isoleucine;

30 X<sup>5</sup> is either valine, leucine, or isoleucine;

X<sup>6</sup> is either lysine, arginine, or histidine;

X<sup>7</sup> is either valine, leucine, or isoleucine;

X<sup>8</sup> is either aspartic acid, glutamic acid, valine, leucine, isoleucine, lysine, arginine, or histidine;

X<sup>9</sup> is either valine, leucine, or isoleucine;

X<sup>10</sup> is either serine, cysteine, threonine, asparagine, glutamine, aspartic acid, or glutamic acid;

each Y<sup>1</sup> is an independently selected amino acid,

5 each Y<sup>2</sup> is an independently selected amino acid,

Z<sup>1</sup> is an optionally present protecting group covalently joined to Y<sup>1</sup>,

Z<sup>2</sup> is an optionally present protecting group covalently joined to Y<sup>2</sup>,

m is from 0 to 300, and

n is from 0 to 300;

10 a pharmaceutically acceptable salt of said polypeptide; and  
a prodrug thereof;

provided that if said compound is said polypeptide then at least one of Z<sup>1</sup> or Z<sup>2</sup> is present.

15 27. The compound of claim 26, wherein m is from 0 to 25, and n is from 0 to 25.

28. The compound of claim 27, wherein said compound is said pharmaceutically acceptable salt.

20 29. A compound selected from the group consisting of:

KGSVVIVGRILSGRK (SEQ. ID. NO. 16),

Ac-GGSVVIVGRILSGRK (SEQ. ID. NO. 18),

GGSVVIVGRILSGRG (SEQ. ID. NO. 19),

25 KKGSVVIVGRILSGRPAIVPRR-NH<sub>2</sub> (SEQ. ID. NO. 20), and

KKGSVVIVGRILSGRPAIVPDRELLYQEFDE (SEQ. ID. NO. 21),

or a pharmaceutically acceptable salt thereof.

30. A nucleic acid comprising a nucleotide sequence encoding for  
30 the HCV inhibitor polypeptide of claim 25.

31. The nucleic acid of claim 30, wherein said nucleic acid is an expression vector.

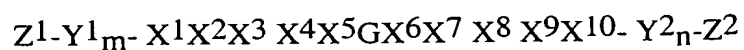


32. A nucleic acid comprising a nucleotide sequence encoding for the polypeptide of claim 26.

33. The nucleic acid of claim 32, wherein said nucleic acid is an expression vector.

34. A pharmaceutical composition for inhibiting HCV replication comprising;  
a pharmaceutically acceptable carrier; and  
an effective amount of a compound selected from the group consisting of:  
an HCV inhibitor polypeptide comprising an NS4A fragment at least about 11 amino acids in length, wherein said fragment can inhibit autocleavage of NS2/3;  
a pharmaceutically acceptable salt of said HCV inhibitor polypeptide; and  
a prodrug thereof.

35. A pharmaceutical composition for inhibiting HCV replication comprising:  
a pharmaceutically acceptable carrier; and  
an effective amount of a polypeptide having the structure:



wherein  $X^1$  is either serine, cysteine, or threonine;  
 $X^2$  is either valine, leucine, or isoleucine;  
 $X^3$  is either valine, leucine, isoleucine, serine, cysteine or threonine;  
 $X^4$  is either valine, leucine, or isoleucine;  
 $X^5$  is either valine, leucine, or isoleucine;  
 $X^6$  is either lysine, arginine, or histidine;  
 $X^7$  is either valine, leucine, or isoleucine;  
 $X^8$  is either aspartic acid, glutamic acid, valine, leucine, isoleucine, lysine, arginine, or histidine;  
 $X^9$  is either valine, leucine, or isoleucine;

X<sup>10</sup> is either serine, cysteine, threonine, asparagine, glutamine, aspartic acid, or glutamic acid;

each Y<sup>1</sup> is an independently selected amino acid,

each Y<sup>2</sup> is an independently selected amino acid,

5 Z<sup>1</sup> is an optionally present protecting group covalently joined to Y<sup>1</sup>,

Z<sup>2</sup> is an optionally present protecting group covalently joined to Y<sup>2</sup>,

m is from 0 to 300, and

n is from 0 to 300;

10 a pharmaceutically acceptable salt of said polypeptide; and  
a prodrug thereof.

36. A pharmaceutical composition for inhibiting HCV replication comprising: a pharmaceutically acceptable carrier; and an effective amount of a nucleic acid encoding for a polypeptide comprising a fragment of NS4A at least about  
15 11 amino acids in length, wherein said fragment can inhibit autocleavage of NS2/3.

37. The composition of claim 36, wherein said nucleic acid is present in an expression vector providing for expression in a human.

20 38. A pharmaceutical composition for inhibiting HCV replication comprising a pharmaceutically acceptable carrier and an effective amount of a nucleic acid encoding for a polypeptide having the structure:



25 wherein X<sup>1</sup> is either serine, cysteine, or threonine;

X<sup>2</sup> is either valine, leucine, or isoleucine;

X<sup>3</sup> is either valine, leucine, isoleucine, serine, cysteine or threonine;

X<sup>4</sup> is either valine, leucine, or isoleucine;

30 X<sup>5</sup> is either valine, leucine, or isoleucine;

X<sup>6</sup> is either lysine, arginine, or histidine;

X<sup>7</sup> is either valine, leucine, or isoleucine;

X<sup>8</sup> is either aspartic acid, glutamic acid, valine, leucine, isoleucine, lysine, arginine, or histidine;

X<sup>9</sup> is either valine, leucine, or isoleucine;

X<sup>10</sup> is either serine, cysteine, threonine, asparagine, glutamine, aspartic acid, or glutamic acid;

each Y<sup>1</sup> is an independently selected amino acid;

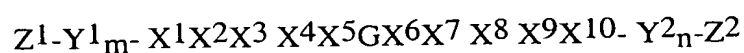
5 each Y<sup>2</sup> is an independently selected amino acid;

m is from 0 to 300; and

n is from 0 to 300.

39. The composition of claim 38, wherein said nucleic acid is  
10 present in an expression vector providing for expression in a human.

40. A method for inhibiting HCV polyprotein processing  
comprising the step of contacting a cell expressing an HCV polypeptide that contains  
at least NS2/3 with an inhibitory polypeptide that either comprises an NS4A fragment  
15 at least about 11 amino acids in length able to inhibit NS2/3 autocleavage or has the  
structure:



20 wherein X<sup>1</sup> is either serine, cysteine, or threonine;

X<sup>2</sup> is either valine, leucine, or isoleucine;

X<sup>3</sup> is either valine, leucine, isoleucine, serine, cysteine or threonine;

X<sup>4</sup> is either valine, leucine, or isoleucine;

X<sup>5</sup> is either valine, leucine, or isoleucine;

25 X<sup>6</sup> is either lysine, arginine, or histidine;

X<sup>7</sup> is either valine, leucine, or isoleucine;

X<sup>8</sup> is either aspartic acid, glutamic acid, valine, leucine, isoleucine, lysine, arginine,  
or histidine;

X<sup>9</sup> is either valine, leucine, or isoleucine;

30 X<sup>10</sup> is either serine, cysteine, threonine, asparagine, glutamine, aspartic acid, or  
glutamic acid;

each Y<sup>1</sup> is an independently selected amino acid,

each Y<sup>2</sup> is an independently selected amino acid,

Z<sup>1</sup> is an optionally present protecting group covalently joined to Y<sup>1</sup>,

Z<sup>2</sup> is an optionally present protecting group covalently joined to Y<sup>2</sup>,  
 m is from 0 to 300, and  
 n is from 0 to 300,

- 5      a pharmaceutically acceptable salt of said inhibitory  
 polypeptide; and  
 a prodrug thereof.

41.      The method of claim 40, wherein said polypeptide is selected  
 from the group consisting of:  
 10      KGSVVIVGRIILSGRK (SEQ. ID. NO. 16),  
 Ac-GGSVVIVGRIILSGRK (SEQ. ID. NO. 18),  
 GGSVVIVGRIILSGRG (SEQ. ID. NO. 19),  
 KKGSVVIVGRIILSGRPAIVPRR-NH<sub>2</sub> (SEQ. ID. NO. 20), and  
 KKGSVVIVGRIILSGRPAIVPDRELLYQEFDE (SEQ. ID. NO. 21),  
 15      or a pharmaceutically acceptable salt thereof.

42.      A method of screening for a compound that inhibits HCV  
 replication or HCV polyprotein processing comprising the steps of:  
 a)      selecting for a compound that binds to the NS4A target  
 20      site using a polypeptide comprising NS2/3 or a binding portion thereof, and  
 b)      measuring the ability of said compound to inhibit HCV  
 replication or HCV polyprotein processing.

43.      The method of claim 42, wherein said method measures the  
 25      ability of said compound to inhibit HCV polyprotein processing.

44.      The method of claim 42, wherein said step (b) is performed in  
 the presence of a non-saturating amount of a NS4A agonist.

- 30      45.      A method of screening for a compound that inhibits HCV  
 replication or HCV polyprotein processing comprising the step of measuring the  
 ability of said compound to inhibit HCV replication or HCV polyprotein processing in  
 the presence of a non-saturating amount of a NS4A agonist.